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Contact: G.E. Walsh, 8-686-9011 (Gulf Breeze, FL)

16 ABSTRACT

Four species of marine unicellular algae were exposed to Kepone in laboratory bioassays. EC50 values after seven days' growth, in mg/liter (ppm), were: Chlorococcum sp., 0.35; Dunaliella tertiolecta, 0.58; Nitzschia sp., 0.60; Thalassiosira pseudonana, 0.60. When exposed to 100 µg/liter (ppb) Kepone for 24 hr, residues associated with the algae, in mg/kg (ppm) wet weight, were: Chlorococcum sp., 80; D. tertiolecta, 23; Nitzschia sp., 41; T. pseudonana, 52.

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a DESCRIPTORS	b IDENTIFIERS/OPEN ENDED TERMS	c COSATI Field/Group

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Short Papers and Notes

Toxicity and Uptake of Kepone in Marine Unicellular Algae[1]

ABSTRACT: Four species of marine unicellular algae were exposed to Kepone in laboratory bioassays. EC50 values after seven days' growth, in mg/liter (ppm), were: *Chlorococcum* sp., 0.33; *Dunaliella tertiolecta*, 0.58; *Nitzschia* sp., 0.60; *Thalassiosira pseudonana*, 0.66. When exposed to 100 mg/liter (ppb) Kepone for 24 hr, residues associated with the algae, in mg/kg (ppm) wet weight, were: *Chlorococcum* sp., 80; *D. tertiolecta*, 33; *Nitzschia* sp., 41; *T. pseudonana*, 52.

Kepone® (decachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalene-2-one) is used for control of banana and potato pests and as an ant and roach killer. The insecticide has been reported in water and biota of the Appomattox and James rivers, Virginia (Environmental Protection Agency 1974). Kepone is structurally similar to the insecticide mirex (dodecachlorooctahydro-1,3,4-metheno-2H-cyclobuta [cd] pentalene) and may be a product of its photodegradation (Ivie *et al.* 1974).

We exposed four species of marine unicellular algae to Kepone to determine its effects on growth and its uptake by phytoplankton. Since algae are food for animals, oxygenate water, and regulate nutrients, an effect on them could affect physical, chemical, and biological properties of aquatic ecosystems.

Materials and Methods

Algae tested were *Chlorococcum* sp. (Milford, Ct.), *Dunaliella tertiolecta* (chlorophyte), *Nitzschia* sp. (UB 648), and *Thalassiosira pseudonana* (bacillariophyte). All were obtained from the Culture Collection of Algae, Indiana University.

In growth studies, algae were grown either in (1) 25 ml of medium in optically matched test tubes shaken at approximately 80 excursions per minute on a New Brunswick Model G shaker fitted with an EziAngle® (New Brunswick Scientific Co., New Brunswick, Connecticut) clamp, or (2) 50 ml of medium in 125-ml Erlenmeyer flasks shaken at approximately 60 excursions per minute. In the former, optical density at 535 nm on a Fisher electrophotometer was determined each day for seven days after inoculation; in the latter, optical density was determined after seven days of growth.

Growth medium was artificial seawater of 30 parts per thousand salinity with full and half-nutrient strengths (Hollister *et al.* 1975). Temperature was 20 ± 0.5°C under 5,000 lux illumination from cool white fluorescent tubes with alternate 12-hr periods of light and darkness. Technical grade Kepone was added in 0.1 ml acetone and 0.1 ml acetone was added to all control cultures. This concentration of acetone did not affect growth. During seven growth tests, each exposure was performed three times. Concentrations that reduced growth by 50 per cent (EC50) after seven days were calculated (American Public Health Association 1971).

In uptake studies, 12 cultures of each alga were grown in 100 ml of full nutrient medium for six days. At that time, cell numbers were between 2×10^6 and 3×10^6 cells/ml, and Kepone, in 0.1 ml acetone, was then added at the nominal concentration of 100 µg/liter. This was well below its solubility limit of 2–4 mg/liter in seawater (May, unpublished). On the seventh day, the algae were harvested by centrifugation, washed four times with uncontaminated medium, and analyzed for kepone by gas chromatography. Each value in Table 1 is the average of 12 replications.

For residue analysis, algae samples were weighed in 125 mm × 15 mm (0.D.) screw-top test tubes and extracted with two 5-ml portions of acetonitrile for 30 sec with a Model PT 10/35 Willems Polytron (Brinkman Instruments, Westbury, New York). The acetonitrile extract was transferred to a 250-ml separatory funnel after centrifugation, and the algae again extracted, first with 5 ml and then with 10 ml of acetone. After each extraction, the tube was centrifuged, and the extract placed in the separatory funnel. Then, 100 ml of 2.0% aqueous sodium sulfate and 10 ml of 1:1 diethyl ether-petroleum ether was added, and the mixture shaken for 1 minute. After the solvent phases separated, the lower aqueous phase was drained into a 250-ml beaker and the upper layer collected in a 25-ml Kuderna-Danish concentrator tube. The ether extraction was repeated three times with 5 ml of 1:1 diethyl ether-petroleum ether. The combined extracts were concentrated to dryness by placing the tube in a water bath at 45°C and evaporating the solvent with a gentle stream of nitrogen. The residue was transferred to a 200 mm × 9 mm (I.D.) Chromaflex® column (Kontes Glass Co., Vineland, N.J.) containing 3.0 gm of Florisil® overlain by 2.0 gm of anhydrous sodium sulfate. The column was first washed with 10 ml of hexane and the residue transferred with four 0.5-ml volumes of 5% diethyl ether in hexane to remove PCB and pesticides. Kepone was removed by a second elution with 40 ml of 15% methanol in benzene. Extracts concentrated or diluted to appropriate volume were analyzed by electron-capture gas chromatography on Varian Aerograph Models 2700 and 1400 Gas Chromatographs equipped with 182 cm × 2 mm (I.D.) glass columns packed with 3% SP2100 and 0.75% SP2250-0.97%

[1] Contribution No. 293 from the Environmental Research Laboratory, Gulf Breeze.

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222–223

TABLE 1. Depression of growth of marine unicellular algae by Kepone and concentrations of Kepone in algae after exposure to 100 µg/l for 24 hours.

Algae	EC50* µg/l	Cell Residue ng/g	Concentration Factor
Chlorococcum sp.	0.35 (0.27–0.40)	80	800
D. tertiolecta	0.58 (0.51–0.64)	23	230
Nitzschia sp.	0.60 (0.53–0.66)	41	410
T. pseudonana	0.60 (0.50–0.70)	53	530

* Range in parentheses.

SP2401 on 100/120 mesh Supelcoport®. The operation conditions were: oven temperature, 182°C; injector temperature, 200°C; detector temperature, 215°C; nitrogen carrier gas flow rate, 25 ml/min. All samples were fortified with an internal standard (dichlorobenzophenone) prior to analysis to evaluate the integrity of results. The average recovery rate of Kepone from fortified algae was 83%. Residue concentrations were calculated on a wet weight basis without correction factor for percentage recovery.

Results

Effects and uptake of Kepone are given in Table 1. Because results from bioassays with full and half-strength nutrient media were identical, the data were combined. Seventh-day EC50 values are representative of effect, because the degree of growth inhibition was constant from day to day. *Chlorococcum* sp. was the most sensitive species; its EC50 values never overlapped those of the others. The EC50 values of the other species were nearly identical.

Chlorococcum sp. also accumulated the greatest amount of Kepone from the medium in the 24-hr exposure, and all differences, except between *Nitzschia* sp. and *T. pseudonana*, are highly significant statistically (t-test for two means, Brownlee 1965).

Discussion

Butler (1964) stated that 1 mg/liter of Kepone reduced carbon fixation by estuarine phytoplankton by 95 per cent after exposure for 4 hr. Our data show that toxicity occurred at much lower concentrations and that algae can accumulate the chemical from water. Since EC50 concentrations were higher than those reported from the Appomattox and James rivers (Environmental Protection Agency 1975), Kepone may have little effect on phytoplankton in the field. However, uptake by algae could result in accumulation at higher trophic levels.

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Gerald E. Walsh, Karen Ainsworth and Albert J. Wilson

Environmental Protection Agency
Environmental Research Laboratory—Gulf Breeze
Gulf Breeze, Florida 32561

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